Human Papillomavirus Type 16 and 18 Variants: Race-Related Distribution and Persistence

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Background: **Analogous to the geographic distribution of variants of human papillomavirus (HPV) types, the distribution and persistence of these variants among infected individuals may be related to the racial composition of a population living in one geographic region.** ***Methods:*** **We studied 1114 women in the United States participating in the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study who were positive for HPV16 and/or HPV18 at enrollment. Race was self-reported. HPV samples were characterized by sequencing the E6 gene and part of the long control region and classified as variants according to established lineages. Subjects were examined for HPV every 6 months for 2 years. All statistical tests were two-sided.** ***Results:*** **HPV18 African variants were predominant in the 97 HPV18-infected African American women (i.e., 62 women or 63.9%, 95% confidence interval [CI] = 53.5% to 73.4%), and European variants were common in the 168 HPV18-infected white women (i.e., 91 women or 54.2%, 95% CI = 46.3% to 61.9%). HPV16 African variants accounted for 43 (26.5%, 95% CI = 19.9% to 34.0%) of the infections in the 162 HPV16-infected African American women but for only 25 (4.3%, 95% CI = 2.8% to 6.3%) in the 584 HPV16-infected white women. The likelihood of remaining HPV18 positive was statistically significantly higher in white women infected with European than in white women infected with African variants (*P* = .04); the reverse was observed in African American women (*P* = .03). A similar pattern was observed for persistence of HPV16 variants, with the likelihood of remaining positive being higher for white women, but lower for African American women, infected with an European variant than with an African variant (*P* = .03 and *P* = .16, respectively).** ***Conclusions:*** **Variants of HPV16 and HPV18 appear to persist longer in a host whose race indicates an ancestral geographic distribution that was once shared with that of the variant—i.e., European variants persist longer in white women, and African variants persist longer in African American women. [J Natl Cancer Inst 2006;98:1045–52]**

Infection with high-risk human papillomavirus (HPV) types, particularly types 16 and 18, causes cervical cancer. To date, nearly 100 HPV types have been identified and characterized *(1)*. For any given HPV type, viral isolates that differ by less than 2% of the DNA sequence for the L1 gene are designated as variants. Variants for a given HPV type appear to segregate geographically *(2–5)*. Although the reasons for geographic segregation are not clear, differences in the adaptation of the virus to the host after many generations of exposure may be one of the factors that contribute to the endemic nature of HPV variants.

It is possible that HPV variants detected within a population may be distributed among the infected individuals whose race indicates an ancestral geographic distribution that was once shared with that of the variant. For example, in a longitudinal study of female university students, nonwhite women were more likely than white women to acquire non-European variants of HPV16 *(6)*. Although the finding was intriguing, it was inconclusive because of the few infected women available for study. Although plentiful data show differences in duration of persistence between types of HPV *(7–10)*, much less is known about persistence of variants, and only a few studies—with inconsistent findings—have been reported *(11–14)*.

In this study, we examined the distribution of HPV16 and HPV18 variants by racial group in many racially diverse women participating in the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion (ASCUS-LSIL) Triage Study (ALTS), a multicenter randomized

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clinical trial designed to evaluate strategies for triaging women with mildly abnormal Pap smears. We evaluated the variant-specific likelihood of becoming viral DNA negative by racial group by testing consecutive cervical specimens from each woman who was monitored every 6 months for 2 years.

Subjects and Methods

Study Subjects

Study subjects were ALTS participants who had HPV16 or HPV18 DNA detected in cervical swab samples at the time of trial enrollment from January 1, 1997, through December 31, 1998, by polymerase chain reaction (PCR)–based reverse line blot hybridization assay *(15)*. Race of the subjects was self-reported as white, African American, Asian/Pacific Islander, or American Indian/Alaskan native. Information on country of birth was not collected. A full description of the design of the ALTS trial and the characteristics of the study population are available elsewhere *(16,17)*. The study protocol was approved by the National Cancer Institute and the local institutional review boards. All participants provided written informed consent.

A total of 1114 women were eligible for this study, including 784 who were positive for HPV16 alone, 268 who were positive for HPV18 alone, and 62 who were positive for both HPV16 and HPV18. Cervical samples taken at enrollment were assayed by PCR-based DNA sequencing for variant characterization, as described below. Fifty HPV16-positive and 53 HPV18-positive women were excluded because of negative sequencing results. Another five women whose race was not ascertained were also excluded, leaving 791 HPV16-positive and 276 HPV18-positive women for these analyses. Parts of the ALTS protocol that are relevant to the current analyses follow.

ALTS participants were randomly assigned to one of three arms (immediate colposcopy, HPV triage, and conservative management). All participants underwent an entry procedure at enrollment that included an interview, a Pap smear, and the detection and typing of HPV DNA. These procedures were followed by a colposcopically directed biopsy examination of visible lesions for all women in the immediate colposcopy arm, all women who were positive for high-risk HPV types in the HPV triage arm, and all women who had a cytologic test result at enrollment of high-grade squamous intraepithelial lesion (HSIL) in the conservative management arm. All women, regardless of study arm, were required to return at 6-month intervals over a 2-year period for cervical cytologic and HPV testing. During follow-up, women were re-referred for colposcopy and biopsy examination if cytologic evidence of HSIL was detected. Women with biopsy-confirmed cervical intraepithelial neoplasia grade 2 or higher (≥CIN2) received appropriate treatment immediately.

Characterization of HPV16 and HPV18 Variants

Cervical swab samples used for this study were generously provided by the ALTS Group ancillary studies committee. A 100-μL aliquot of each sample was digested with proteinase K (200 μg/mL) in 0.1% Laureth-12 at 56 °C for 1 hour. The digests were precipitated with 1.0 mL of absolute ethanol containing 0.67 M ammonium acetate overnight at −20 °C and then centrifuged for 30 minutes at 13 000*g* at 4 °C. DNA pellets were rinsed with 70% ethanol twice, dried, and resuspended in 30 μL of TE (10 mM Tris, pH 8.0, and 1 mM EDTA).

Sequence variation at HPV16 nucleotide positions 7723 to 567 or at HPV18 positions 7489 to 587 (corresponding to the 3′ part of the long control region and the entire E6 region) was determined by PCR-based DNA sequencing. The fragments of target genes were generated by use of one pair of primers for HPV16 (forward, 5′-AGGCACATATTTTTGGCTTGTT-3′; reverse, 5′-TTCATG CAATGTAGGTGTATCTCC-3′) and the other pair for HPV18 (forward, 5′-GTTGCCTTTGGCTTATGTCTG-3′; reverse, 5′-TT GCCTTAGGTCCATGCATAC-3′). PCR products were isolated on 2% agarose gel and then purified with a QIAEX II gel extraction kit (QIAGEN, Valencia, CA). Purified DNA template (30 ng) was added to the sequencing reaction by use of a Big Dye Terminator Cycle Sequencing kit according to the manufacturer's protocol (Applied Biosystems, Foster City, CA). The reactions were performed from both 5′–3′ and 3′–5′ directions with the above external primers plus one pair of internal primers for HPV18 (forward, 5′-AATACTATGGCGCGCTTTGA-3′; reverse, 5′-TGTCTTG CAGTGAAGTGTTCAG-3′). DNA sequences were analyzed with the Sequencer package (Gene Codes Corp., Ann Arbor, MI).

Variants were compared with the prototype sequences of HPV16 or HPV18 *(18–20)* and then assigned to a lineage on the basis of their similarity to known variants. An analysis of the 751-base-pair fragments of HPV16 revealed 144 variants. By use of a lineage classification system developed on the basis of geographic relatedness *(3,4)*, these variants were categorized as European, Asian, North American, Asian American, African 1 (Af1), and African 2 (Af2). Seventy-four HPV18 variants were identified in an analysis of the 956-base-pair fragments and then further classified by their sequences as European, Asian American, and African variants *(5,21)*.

Statistical Analyses

Kaplan–Meier analyses were performed to estimate the likelihood of women who had an HPV infection detected at enrollment becoming negative for viral DNA. Time to an event was defined as the time between enrollment and the first visit at which a subject became negative (unless otherwise specified). Those women who completed the follow-up without clearing their infection or were lost to follow-up were censored at their last available visit. In an initial analysis, all women with biopsy-confirmed ≥CIN2 at enrollment or during follow-up were censored at the time of initial diagnosis. Because women with ≥CIN2, if not treated, might have had an infection with a prolonged duration, we conducted a second analysis, in which those with ≥CIN2 who were positive for the HPV variant at the time of diagnosis were treated as being positive through the last available visit. A log-rank test *(22)* was used to assess the overall likelihood of becoming negative for viral DNA between the HPV variants by racial group. Because the visits were scheduled every 6 months, the events of viral DNA regression were not ascertained in continuous time; most of them were detected around the scheduled time. For naturally fitting data with discrete follow-up times, a Poisson regression model *(23)* was used to estimate the relative risk (RR) for becoming negative for viral DNA. The events and person-months of observations were grouped into four categories centered at each of the scheduled visits (<9, ≥9 to <15, ≥15 to <21, and ≥21 months). The statistical significance of the main effect and interaction between the variants and race was assessed with a likelihood-ratio test. The proportional

hazards assumption was examined by testing the statistical significance of interaction between the variants and time intervals. The mean length of follow-up and the mean age at enrollment by HPV variant groups were compared by use of a Student's *t* test. A departure of normality of the distribution was assessed by a goodness-of-fit test. A chi-square, or Fisher's exact test when appropriate, was used to compare distribution of the variants by racial group and percentage of women who did not return for follow-up by HPV variant and racial group. Bonferroni corrections were made to adjust for multiple comparisons of distribution of the variants by racial group. All statistical tests were two-sided.

Results

Distribution of HPV Variants by Racial Group

Of the 1025 women included in this study, 719 were white, 251 were African American, 36 were Asian/Pacific Islander, and 19 were American Indian/Alaskan. The mean (± standard deviation [SD]) age at enrollment was 24.8 (±5.8) years. HPV18 variants were studied in samples from 276 women, with European variants detected in 119 (43.1%, 95% confidence interval [CI] = 37.2% to 49.2), with African variants detected in 79 (28.6%, 95% CI = 23.4% to 34.3%), and with Asian American variants detected in 78 (28.3%, 95% CI = 23.0% to 34.0%) of the 276 samples. As shown in Table 1, HPV18 African variants were predominant in the 97 HPV18-infected African American women (i.e., 62 women or 63.9%, 95% CI = 53.5% to 73.4%), and European variants were common in the 168 HPV18-infected white women (i.e., 91 women or 54.2%, 95% CI = 46.3% to 61.9%). The difference in overall distribution of HPV18 variants between white and African American women was statistically significant ($P<.001$). The overall distribution of HPV18 variants in American Indian/Alaskan women and Asian/Pacific Islander women was similar to that in white women but differed substantially from that in African American women ($P = .006$).

HPV16 variants were evaluated in samples from 791 women, with HPV16 European variants detected in 649 (82.0%, 95% CI = 79.2% to 84.7%), Asian variants detected in six (0.8%, 95% CI = 0.3% to 1.6%), North American variants detected in six (0.8%, 95% CI = 0.3% to 1.6%), Asian American variants detected in 61 (7.7%, 95% CI = 5.9% to 9.8%), Af1 variants detected in 36 (4.6%, 95% CI = 3.2% to 6.2%), and Af2 variants detected in 33 (4.2%, 95% CI = 2.9% to 5.8%) of the 791 samples. Because of the limited number of infections, Asian and North American variants were grouped with Asian American and designated as Asian American–positive variants. To make the classification systems for HPV16 variants and HPV18 variants comparable to each other, we grouped Af1 and Af2 variants together in the initial analyses and named them as African variants. HPV16 African variants accounted for 43 (26.5%, 95% CI = 19.9% to 34.0%) of the infections in the 162 HPV16-infected African American women but for only 25 (4.3%, 95% CI = 2.8% to 6.3%) in the 584 HPV16-infected white women (Table 1). The overall distribution of HPV16 variants in African American women differed statistically significantly from that observed in white women ($P<.001$) and in American Indian/Alaskan women or Asian/Pacific Islander women ($P<.001$). Although infections with HPV16 European variants were predominant in all three racial groups, African variants were relatively more common in African American women and Asian American–positive variants were relatively more common in American Indian/Alaskans or Asian/Pacific Islanders.

Given the apparent race-associated difference in distribution of HPV variants, we next examined the association between a subject's race and the persistence of HPV variants. Because of the relatively few American Indian/Alaskans and Asian/Pacific Islanders were positive for HPV16 or HPV18, this analysis was confined to white and African American women who were positive for either European or African variants.

Persistence of HPV18 Variants by Racial Group

Of the 191 white or African American women with either HPV18 European or African variants, nine were not followed and therefore were excluded from the analysis of viral persistence. The percentage of loss to follow-up was 4.7% for both white (n = 106) and African American (n = 85) women, 5.3% for women with HPV18 European variants (n = 114), and 3.9% for women with HPV18 African variants (n = 77), respectively ($P = .74$). Status of ≥CIN2 was histologically confirmed in 34 women at enrollment, in four during follow-up, and in 23 at the end of the study. The mean (±SD) length of follow-up (without taking censoring of ≥CIN2 into account) was 23.1 (±5.1) months for

Table 1. Distribution of human papillomavirus (HPV) type 16 and 18 variants by racial group*

Strain and racial group of women	E variants No. (%, 95% CI)	Af variants No. (%, 95% CI)	AA(+) variants No. (%, 95% CI)	*P*†	*P* value‡
HPV18					
White (n = 168)	91 (54.2, 46.3 to 61.9)	15 (8.9, 5.1 to 14.3)	62 (36.9, 29.6 to 44.7)		
Africa American (n = 97)	23 (23.7, 15.7 to 33.4)	62 (63.9, 53.5 to 73.4)	12 (12.4, 6.6 to 20.6)	<.001	
Others§ (n = 11)	5 (45.5, 16.7 to 76.6)	2 (18.2, 2.3 to 51.8)	4 (36.4, 10.9 to 69.2)	.52	.006
HPV16					
White (n = 584)	505 (86.5, 83.4 to 89.1)	25 (4.3, 2.8 to 6.3)	54 (9.2, 7.0 to 11.9)		
Africa American (n = 162)	108 (66.7, 58.8 to 73.9)	43 (26.5, 19.9 to 34.0)	11 (6.8, 3.4 to 11.8)	<.001	
Others‖ (n = 45)	36 (80.0, 65.4 to 90.4)	1 (2.2, 0.1 to 11.8)	8 (17.8, 8.0 to 32.1)	.17	<.001

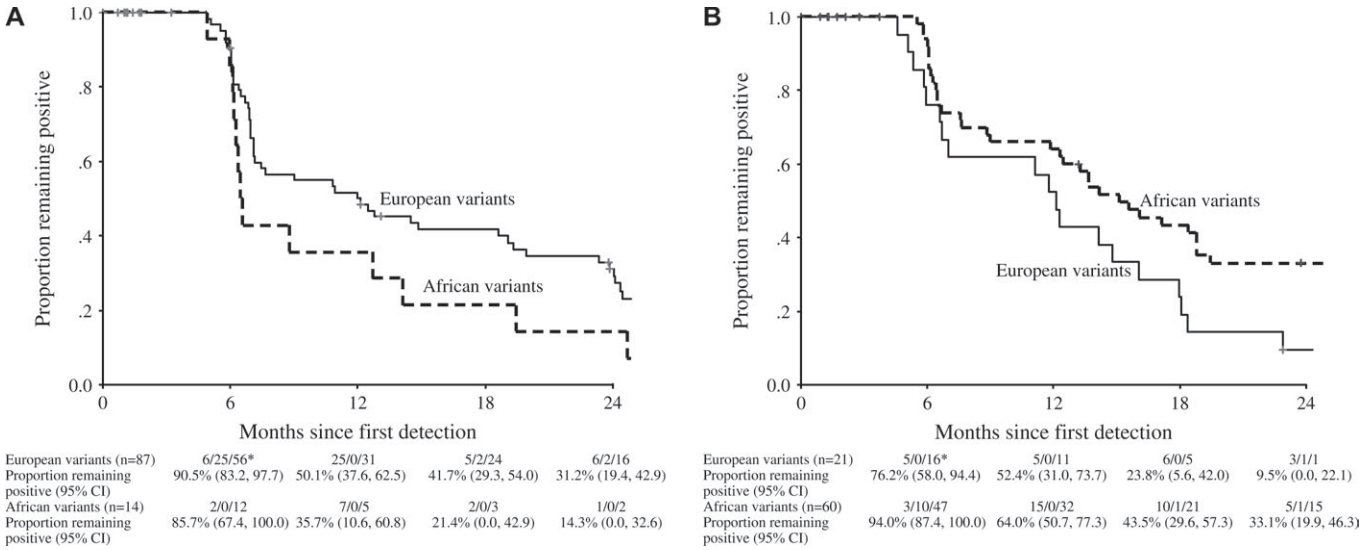
*E = European; Af = African; AA = Asian American; CI = confidence interval.

†Compared with the variant distribution in white women by a chi-square or Fisher's exact test, when appropriate. Bonferroni corrections were made to adjust for multiple comparisons of distribution of the variants by racial group, resulting in a cutoff *P* value of .017 to determine statistical significance. All statistical tests were two-sided.

‡Compared with the variant distribution in African American women by Fisher's exact test.

§Including four American Indian/Alaskan women and seven Asian/Pacific Islander women.

‖Including 15 American Indian/Alaskan women and 30 Asian/Pacific Islander women.



European variants (n=87)	6/25/56*	25/0/31	5/2/24	6/2/16
Proportion remaining positive (95% CI)	90.5% (83.2, 97.7)	50.1% (37.6, 62.5)	41.7% (29.3, 54.0)	31.2% (19.4, 42.9)
African variants (n=14)	2/0/12	7/0/5	2/0/3	1/0/2
Proportion remaining positive (95% CI)	85.7% (67.4, 100.0)	35.7% (10.6, 60.8)	21.4% (0.0, 42.9)	14.3% (0.0, 32.6)

European variants (n=21)	5/0/16*	5/0/11	6/0/5	3/1/1
Proportion remaining positive (95% CI)	76.2% (58.0, 94.4)	52.4% (31.0, 73.7)	23.8% (5.6, 42.0)	9.5% (0.0, 22.1)
African variants (n=60)	3/10/47	15/0/32	10/1/21	5/1/15
Proportion remaining positive (95% CI)	94.0% (87.4, 100.0)	64.0% (50.7, 77.3)	43.5% (29.6, 57.3)	33.1% (19.9, 46.3)

Fig. 1. Proportion of women remaining positive for human papillomavirus (HPV) DNA from the time of enrollment among women infected with an HPV18 variant by racial group. (**A**) White women. (**B**) African American women. Women with biopsy-confirmed cervical intraepithelial neoplasia grade 2 or higher (≥CIN2) were censored at the time of initial diagnosis. Estimates were determined with a Kaplan–Meier analysis. European variants are shown with a **solid line**; African variants are shown with a **dashed line**. The *P* value for the overall likelihood of remaining positive for viral DNA between the European and African variants was .04 among white women and .03 among African American women. *, Number becoming negative/number censored during the interval/number remaining positive at the beginning of the interval and proportion remaining positive, with 95% confidence intervals in parentheses, are shown at 6, 12, 18, and 24 months of follow-up.

women with European variants and 24.1 (±3.7) months for those with African variants ($P = .15$). The mean (±SD) age of women with European and African variants was 25.1 (±5.9) and 25.5 (±5.4) years, respectively ($P = .61$).

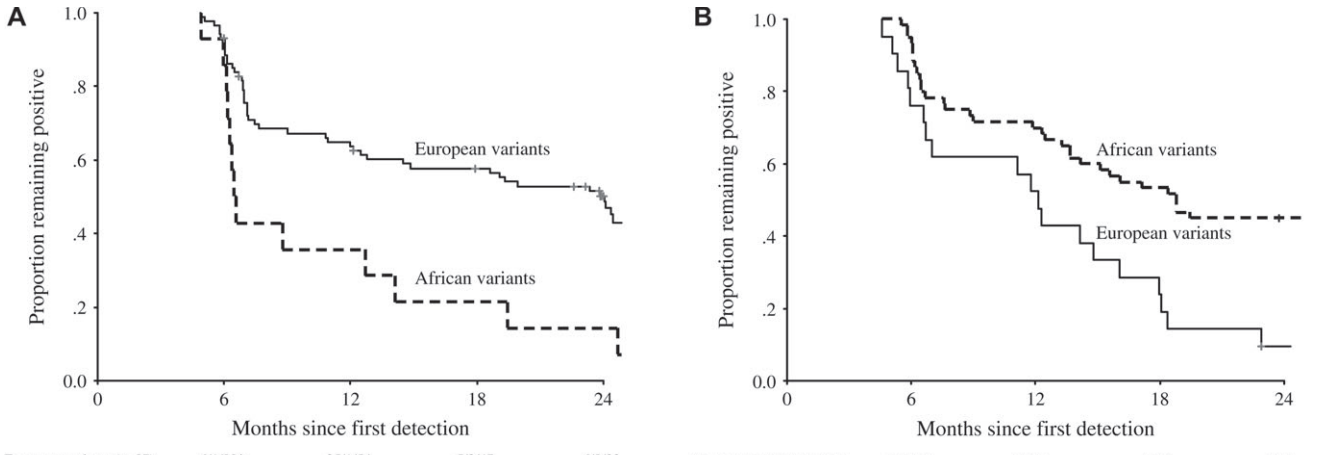
As shown in Fig. 1, after censoring women who were identified with ≥CIN2 at enrollment or during follow-up, the overall likelihood of remaining positive for viral DNA was statistically significantly higher for white women with HPV18 European variants than for white women with HPV18 African variants (log-rank test, $P = .04$). This trend was reversed in African American women; i.e., the overall likelihood of remaining positive for viral DNA was statistically significantly lower for African American women with HPV18 European variants than for African American women with HPV18 African variants (log rank test, $P = .03$). The mean time to HPV18 DNA negativity from the date of enrollment was 15.0 months (95% CI = 12.8 to 17.2 months) and 11.0 months (95% CI = 7.2 to 14.7 months) for white women with HPV18 European and African variants, respectively, and 12.6 months (95% CI = 9.9 to 15.4 months) and 17.1 months (95% CI = 14.3 to 19.9 months) for African American women with HPV18 European and African variants, respectively.

To verify the robustness of the results, we performed a second analysis in which women with biopsy-confirmed ≥CIN2 at enrollment or during follow-up were arbitrarily treated as being positive through the last available visit if they had viral DNA detected at the time of histologic diagnosis. Again, the likelihood of persistent detection of viral DNA remained statistically significantly higher in white women with HPV18 European variants than in white women with HPV18 African variants (Fig. 2, A, log rank test, $P<.001$), but this likelihood was statistically significantly lower in African American women with HPV18 European variants than in African American women with African variants (Fig. 2, B, log rank test, $P = .002$). Because some women had a negative test result between their HPV18-positive visits, determined by the PCR-based reverse line blot hybridization assay, we conducted an analysis that treated women with one negative test as being positive. Results of this analysis, however, did not substantially alter the race-associated difference in persistence of HPV18 variants (data not shown). To address the concerns of clustering of the events and discrete nature of the follow-up times, the relative risk of becoming negative for viral DNA was further assessed by use of Poisson regression analyses. Again, infections with HPV18 African variants were more likely to resolve than infections with European variants in white women (RR = 2.63, 95% CI = 1.45 to 4.74) but less likely to resolve in African American women (RR = 0.50; 95% CI = 0.29 to 0.87). The likelihood of persistence by HPV18 variants between the racial groups was statistically significantly different ($P<.001$).

Persistence of HPV16 Variants by Racial Group

Of the 681 white or African American women with an HPV16 European or African variant, 57 were excluded from the analysis of viral persistence because they did not have a follow-up visit. No statistically significant differences were found in percentages of loss to follow-up between 530 white and 151 African American women (8.9% versus 6.6%; $P = .51$) and between the 613 women with HPV16 European and the 68 with HPV16 African variants (8.6% versus 5.8%; $P = .64$). Status of ≥CIN2 was histologically confirmed in 243 women at enrollment, in 45 during follow-up, and in 57 at the end of the study. The mean (±SD) length of follow-up (without censoring women with ≥CIN2) was 23.1 (±5.4) months and 23.7 (±3.2) months for women with HPV16 European and HPV16 African variants, respectively ($P = .40$). The mean (±SD) age of women with HPV16 European and HPV16 African variants was 25.1 (±6.0) years and 23.8 (±5.7) years, respectively ($P = .12$).

When women with ≥CIN2 at enrollment or during follow-up were censored at the time of initial diagnosis, the overall likelihood of remaining positive was statistically significantly higher in white women with HPV16 European variants than in those

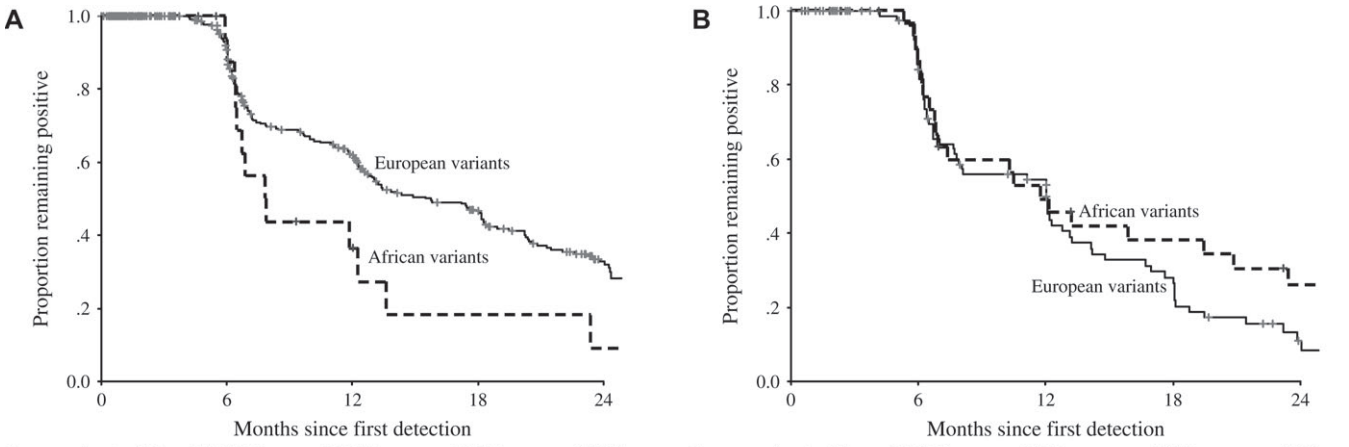


European variants (n=87)	6/1/80*	25/1/54	5/2/47	6/8/33
Proportion remaining positive (95% CI)	93.1% (87.8, 98.4)	63.7% (53.5, 74.0)	57.8% (47.2, 68.3)	50.2% (39.4, 60.9)
African variants (n=14)	2/0/12	7/0/5	2/0/3	1/0/2
Proportion remaining positive (95% CI)	85.7% (67.4, 100.0)	35.7% (10.6, 60.8)	21.4% (0.0, 42.9)	14.3% (0.0, 32.6)

European variants (n=21)	5/0/16*	5/0/11	6/0/5	3/1/1
Proportion remaining positive (95% CI)	76.2% (58.0, 94.4)	52.4% (31.0, 73.7)	23.8% (5.6, 42.0)	9.5% (0.0, 22.1)
African variants (n=60)	3/0/57	15/0/42	10/0/32	5/2/25
Proportion remaining positive (95% CI)	95.0% (89.5, 100.0)	70.0% (58.4, 81.6)	53.3% (40.7, 66.0)	45.0% (32.4, 57.6)

Fig. 2. Proportion of women remaining positive for human papillomavirus (HPV) DNA from the time of enrollment among women infected with an HPV18 variant by racial group. (**A**) White women. (**B**) African American women. Estimates were determined with a Kaplan–Meier analysis. Women with biopsy-confirmed cervical intraepithelial neoplasia grade 2 or higher (≥CIN2) were treated as being positive through the last available visit. European variants are shown with a **solid line**; African variants are shown with a **dashed line**. The *P* value for the overall likelihood of remaining positive for viral DNA between the European and African variants was less than .001 among white women and .002 among African American women. *, Number becoming negative/number censored during the interval/number remaining positive at the beginning of the interval and proportion remaining positive, with 95% confidence intervals in parentheses, are shown at 6, 12, 18, and 24 months of follow-up.

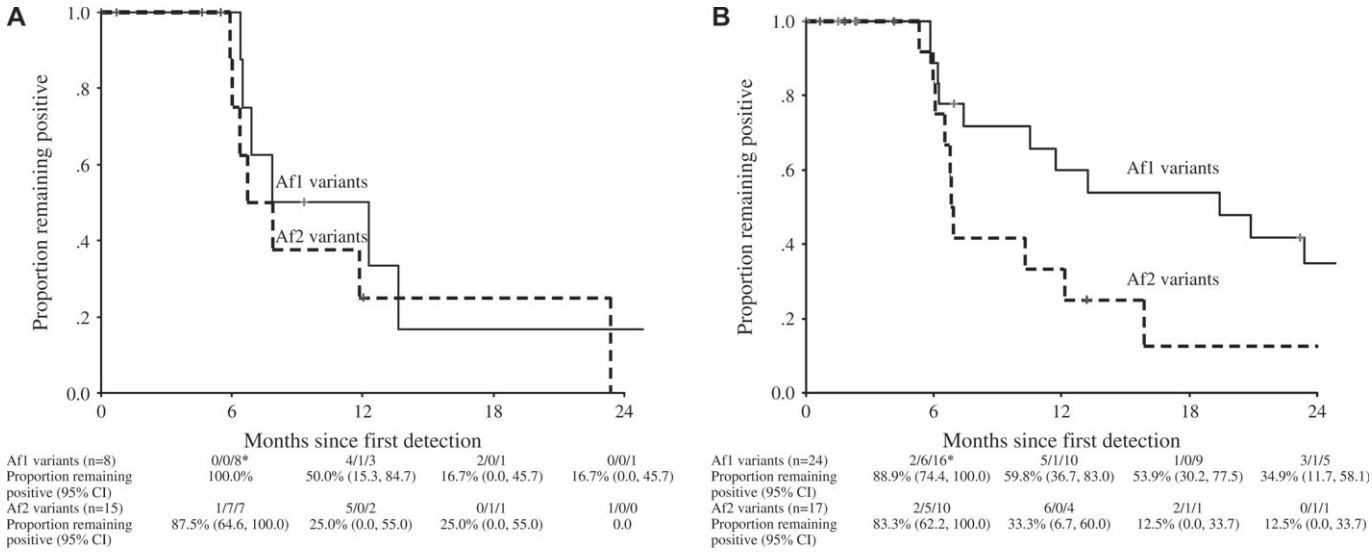
with HPV16 African variants (Fig. 3, A, log-rank test, $P = .03$). Among African American women, the likelihood of remaining positive for viral DNA appeared to be higher in those with HPV16 African variants than in those with HPV16 European variants, but the difference was not statistically significant (Fig. 3, B, log-rank test, $P = .16$). The mean time to HPV16 DNA negativity from the date of enrollment was 17.2 months (95% CI = 15.8 to 18.6 months) and 11.4 months (95% CI = 8.0 to 14.9 months) for white women with HPV16 European variants and HPV16 African variants, respectively. Among African American women, this value was 12.9 months (95% CI = 11.2 to 14.5 months) for those with HPV16 European variants and 15.0 months (95% CI = 11.7 to 18.3 months) for those with HPV16 African variants. Again, neither treating ≥CIN2 as being positive through the last available visit nor treating one intercurrent negative test as being positive substantially changed the race-associated differences in persistence of HPV16 variants (data not shown). Infections with HPV16 African, compared with European, variants were more likely to resolve in white women (RR = 1.94, 95% CI = 1.10 to 3.41) but less likely to resolve in African American women



European variants (n=460)	24/200/236*	69/28/139	33/15/91	24/26/41
Proportion remaining positive (95% CI)	90.9% (87.5, 94.4)	62.3% (56.2, 68.4)	46.6% (40.0, 53.1)	32.7% (26.1, 39.4)
African variants (n=23)	1/7/15	9/1/5	2/1/2	1/0/1
Proportion remaining positive (95% CI)	93.8% (81.9, 100.0)	36.5% (12.4, 60.6)	18.2% (0.0, 39.8)	9.1% (0.0, 25.7)

European variants (n=100)	12/25/63*	23/5/35	16/1/18	10/4/4
Proportion remaining positive (95% CI)	84.2% (76.0, 92.4)	52.9% (41.5, 64.3)	28.1% (17.3, 38.8)	11.0% (2.9, 19.2)
African variants (n=41)	4/11/26	11/1/14	3/1/10	3/1/6
Proportion remaining positive (95% CI)	86.7% (74.5, 98.8)	49.3% (31.2, 67.4)	38.1% (20.2, 56.0)	26.1% (9.4, 42.8)

Fig. 3. Proportion of women remaining positive for human papillomavirus (HPV) DNA from the time of enrollment among women infected with HPV16 by racial group. (**A**) White women. (**B**) African American women. Estimates were determined with a Kaplan–Meier analysis. European variants are shown with a **solid line**; African variants are shown with a **dashed line**. We censored women with biopsy-confirmed cervical intraepithelial neoplasia grade 2 or higher (≥CIN2) at the time of initial diagnosis. The *P* value for the overall likelihood of remaining positive between the European and African variants was .03 among white women and .16 among African American women. *, Number becoming negative/number censored during the interval/number remaining positive at the beginning of the interval and proportion remaining positive, with 95% confidence intervals in parentheses, are shown at 6, 12, 18, and 24 months of follow-up.



Af1 variants (n=8)	0/0/8*	4/1/3	2/0/1	0/0/1
Proportion remaining positive (95% CI)	100.0%	50.0% (15.3, 84.7)	16.7% (0.0, 45.7)	16.7% (0.0, 45.7)
Af2 variants (n=15)	1/7/7	5/0/2	0/1/1	1/0/0
Proportion remaining positive (95% CI)	87.5% (64.6, 100.0)	25.0% (0.0, 55.0)	25.0% (0.0, 55.0)	0.0

Af1 variants (n=24)	2/6/16*	5/1/10	1/0/9	3/1/5
Proportion remaining positive (95% CI)	88.9% (74.4, 100.0)	59.8% (36.7, 83.0)	53.9% (30.2, 77.5)	34.9% (11.7, 58.1)
Af2 variants (n=17)	2/5/10	6/0/4	2/1/1	0/1/1
Proportion remaining positive (95% CI)	83.3% (62.2, 100.0)	33.3% (6.7, 60.0)	12.5% (0.0, 33.7)	12.5% (0.0, 33.7)

Fig. 4. Proportion of women remaining positive for human papillomavirus (HPV) DNA from the time of enrollment among women who were infected with HPV16 by racial group. (**A**) White women. (**B**) African American women. Estimates were determined with a Kaplan–Meier analysis. Women with biopsy-confirmed cervical intraepithelial neoplasia grade 2 or higher (≥CIN2) were censored at the time of initial diagnosis. HPV16 African 1 (Af1) variants are shown with a **solid line**; African 2 (Af2) variants are shown with a **dashed line**. The *P* value for the overall likelihood of remaining positive between the Af1 and Af2 variants was .42 among white women and .06 among African American women. *, Number becoming negative/number censored during the interval/number remaining positive at the beginning of the interval and proportion remaining positive, with 95% confidence intervals in parentheses, are shown at 6, 12, 18, and 24 months of follow-up.

(RR = 0.76, 95% CI = 0.47 to 1.24). The difference between the racial groups was statistically significant ($P = .014$).

Among those with HPV16 African variants, eight (34.8%) of the 23 white women and 24 (58.5%) of the 41 African American women were positive for Af1 variants ($P = .07$). As shown in Fig. 4, B, African American women with Af1 variants appeared to resolve their infections more slowly than did those with Af2 variants (log rank test, $P = .06$). The mean time to HPV16 DNA negativity was 17.6 months (95% CI = 13.2 to 22.0 months) for African American women with HPV16 Af1 variants and 11.4 months (95% CI = 6.1 to 16.8 months) for African American women with Af2 variants. There was no appreciable difference in the likelihood of remaining positive for infections with HPV16 Af1 and Af2 variants in white women (Fig. 4, A, log rank test, $P = .42$). The mean time to HPV16 DNA negativity from the date of enrollment was 12.0 months (95% CI = 7.2 to 16.8 months) and 11.4 months (95% CI = 6.1 to 16.8 months) for white women with HPV16 Af1 and Af2 variants, respectively.

Discussion

In this study of racially diverse women residing in the United States, we found that infection with European variants was most common in HPV18-positive white women and infection with African variants was most common in HPV18-positive African American women. Similarly, the proportion of African variants was statistically significantly higher in HPV16-positive African American women than in HPV16-positive white women. In African American women infected with HPV16 African variants, most infections were with Af1 variants. This race-related distribution of HPV16 and HPV18 variants is consistent with previous findings of geographic-related distributions, whereby the European variants were predominant in populations in Europe and African variants were predominant in Africa *(2–5)*. Furthermore, the Af1 variant accounted for most of the HPV16 infections in the African population *(4)*.

One interpretation of our results is that the race-associated variant distribution may simply reflect long-term sexual mixing patterns in the population, whereby sexual activities more commonly occur between members of the same race than between members of different races. Alternatively, genetic factors may preferentially predispose women to establish and/or retain infection with particular HPV variants. A higher proportion of certain variants in HPV infections detected at enrollment could result from increased acquisition and/or prolonged persistence. Our previous cohort study of the natural history of HPV16 variant infections *(6)* showed that the risk of incident infection with non-European variants was statistically significantly higher in non-white women than in white women, a finding that supports the role of acquisition as an explanation for the race-associated variant distribution. However, it is unclear whether this pattern would be due to race-related differences in the risk of exposure to particular variants or to differences in predispositions for establishing infections with different variants.

As an important contribution to the understanding of viral–host interactions, this study demonstrated that infections with European variants, compared with infections with African variants, were less likely to resolve in white women but more likely to resolve in African American women. It also showed that the likelihood of remaining positive was higher for African American women with Af1 variants than for African American women with Af2 variants. The difference was not explained by the length of follow-up or by age at enrollment, a factor thought to be related to viral persistence *(24)*. Ascertainment bias was not an issue because assessing HPV variants was done without information on viral DNA persistence. Therefore, the difference may reflect host-related abilities of the variants to establish a persistent infection.

One issue complicating our finding of race-associated viral DNA persistence is that the study included a substantial number of women who received a therapeutic procedure for their biopsy-confirmed ≥CIN2. As indicated previously *(25,26)*, viral persistence is altered by a treatment procedure such as laser conization

and loop electrosurgical excision procedure; the latter procedure was used almost exclusively in the ALTS trial. Indeed, in the ALTS study, women who were treated for ≥CIN2 at enrollment were more likely to resolve their infections by the subsequent follow-up visit than were those without ≥CIN2 at enrollment who did not receive the treatment. Clearly, the results would have been biased if those with and without ≥CIN2 had been treated uniformly in the analysis. Also, restricting the analysis to women without ≥CIN2 may not be appropriate because this restriction actually removes those who are likely to have a persistent infection. Because such a disturbance to viral DNA persistence (which is the result of the presence of cervical lesions and their treatment) is unavoidable in observational studies, we treated women with biopsy-confirmed ≥CIN2 as either censored at the time of initial diagnosis or positive through the last available visit. Both analyses yielded equivalent results. Further analyses of viral DNA clearance at the first follow-up visit suggest that the impact of treatment on clearance of viral infection was not related to race or variant (data not shown). Thus, it is unlikely that treatment biased the finding of race-associated viral DNA persistence.

Some women had a negative HPV test result between two positive visits. In our main analyses, the event of viral clearance was defined as the first negative result. However, negativity between the positive visits may be due to a natural fluctuation in the level of viral DNA or variability in the sampling procedure. Biases could have been introduced if these differences were differentially related to the variants. However, when we treated intercurrent negative visits as positive, the estimates remained similar. Finally, we are aware that, because the study protocol required testing for HPV DNA at each 6-month study visit, most of the regression events occurred around the scheduled time. To assess whether, and to what extent, the estimates were influenced by the clustering of the events and the almost discrete nature of the follow-up times, the data were further analyzed with a Poisson regression. The consistency of the results from the Poisson regression and those from the log-rank test suggests that our findings are robust.

Studies of persistence of HPV variants are rare, and findings are inconsistent *(11–14)*. These studies varied by classification of variants and definitions of persistence. Most of these studies dichotomized persistent status only by the presence or absence of HPV DNA in one or more consecutive visits. One recent study *(13)* reported that, relative to nononcogenic HPV types, the risk for viral persistence was somewhat higher for non-European than for European variants; this analysis, however, did not separate HPV16 and HPV18 infections, and the difference between two variant groups was not substantial. Our previous data *(6)* from a cohort of female university students with incident HPV16 infections provided no evidence of a difference in persistent detection of European and non-European variants. However, the analysis in our previous study was not stratified by racial group because of the small number of study subjects. Until now, data on the race-associated persistence of HPV variants have been unavailable. As suggested by this study, differences in viral DNA persistence between the variants may be substantially attenuated if race is not taken into account.

Viral–host interactions are believed to play a crucial role in clearing HPV DNA. The race-associated difference in persistence of HPV variants may result from decreased cellular immunity, perhaps through a specific immune evasion mechanism between the variant and the host that would reduce an otherwise effective T-cell response and make it less effective in clearing the infections. In this study, we examined sequence variation in the part of viral genome. It is not known which viral antigens are responsible for difference in clearance because those may be linked polymorphisms throughout the genome. Because persistent, compared with transient, HPV infection increases the risk of cervical diseases, the findings of the race-associated difference in viral DNA persistence between the variants may explain in part our observation of variant-related risk of cervical neoplasia (Xi LF, Koutsky LA, Hildesheim A, Galloway DA, Wheeler CM, Winer RL, et al., unpublished data).

Our study has several limitations. First, women included in this study were those who were positive for HPV16 or HPV18 at enrollment without distinction between new and previously existing infections. However, there is no reason to believe that African variants detected in white women had already persisted longer than European variants at the time of enrollment or that those in African American women had been acquired recently. Usually, previously existing infections are more likely than incident infections to represent those with a prolonged duration. Second, we observed that approximately 8% of white and African American women with HPV16 European or African variants and 5% of those with HPV18 European or African variants failed to return for follow-up. Biases could have been introduced if loss to follow-up had been differentially related to viral clearance between the variants by racial group. However, women who were not followed up did not differ from those who were followed up with respect to race and variants. Also, there was no apparent difference in mean length of follow-up by HPV variant and racial group. Third, we recognize that the use of prevalent cohorts may result in some biases in the estimated relative hazards of viral DNA changing from detectable to undetectable status; nevertheless, the comparison of groups is still valid because the potential influence is likely to be nondifferential *(27)*.

In summary, our data indicate that the distribution of HPV16 and HPV18 variants appears to be related to race among women presently living in the same geographic region. Given that women with persistent, compared with transient, HPV16 or HPV18 infections are at increased risk of cervical cancer, future studies should be conducted to examine possible mechanisms involving variant-specific immune evasion and their potential clinical and therapeutic implications.

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Notes

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